Next-Generation Probiotics: health-promoting bacteria of the human gut

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Abstract

In recent years, a vast number of human diseases have been correlated with gut microbiota dysbiosis. The development of modern methods in molecular microbiology, such as the culturomics approach, as well as various multi-omics methods like next generation sequencing, transcriptomics and metabolomics analysis, coupled with large data sets correlation analysis, enabled the cultivation and characterization of novel anaerobic hitherto uncultivated Next-Generation Probiotics. In addition, the results of host-microbe interactions studies helped to reveal the mechanisms involved in the beneficial effects of Next-Generation Probiotics. Eventually, the obtained data on Next-Generation Probiotics will help to broaden the scientific knowledge on these bacteria, in terms of both their safety and health-promoting effects, unravel opportunities for the development of novel therapeutic strategies for prevention and treatment of tumors, metabolic, neuropsychiatric and other diseases, with the aim of relieving the symptoms of the diseases and increasing the quality of life for patients and their families. So far, the best characterized probiotics of the new generation are *Akkermansia muciniphila*, *Faecalibacterium prauznitzii* and *Bacteroides fragilis.*

Key words: Next-Generation Probiotics, *Faecalibacterium prauznitzii*, *Akkermansia muciniphila*, *Bacteroides fragilis*

Introduction

The consumption of artisanal dairy products has been previously correlated with human longevity, and this observation opened the era of probiotics that are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Probiotics were shown to have beneficial effects on the gut, metabolism, immune system and functioning of the nervous system (1–6). Commercial probiotics are represented predominantly with different species of *Lactobacillus* sp. and *Bifidobacterium* sp., commonly found in the human intestinal tract and dairy products. The probiotic effects of these bacteria are most frequently based on their anti-microbial effects, thus providing help to the host in dealing with different infections, as well as on their contribution in food digestion, thereby helping the host in food processing (7).

The gut microbiota represents a complex microbial community comprising 10^{10}-10^{14} cells of archaea, bacteria, eukarya, viruses, and bacteriophages, which started a new era of research directed towards deciphering host-gut microbiota interactions, providing the opportunity for discovering new commensal bacteria. Gut microbiota evolved together with their host, conveying mutual beneficial effects that are a prerequisite to the host's wellbeing (8). However, infection, inflammation, diet, stress, and similar circumstances could have an impact on gut microbiota diversity and composition, causing dysbiosis and diseases (9, 10). Nowadays, the development of culture-independent high-throughput molecular methods based on 16S rRNA next generation sequencing (NGS), together with developments in bioinformatics, has significantly improved gut microbiota research and enabled the precise identification of distinctive taxa from phylum to the species level (11). Overall, gut microbiota composition is dominated by five phyla with 90% of phylotypes identified in *Bacteroidetes* and *Firmicutes*, and followed by less abundant *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* (12). Widespread use of NGS created a firm basis for gut microbiota research where the correlations between specific bacterial taxa and various diseases versus a healthy state are evaluated. Although there are a number of research reports related to the differences in diversity and composition between healthy subjects and various patients' groups**,** studies on metagenomics analysis, host–microbiota cross-talk and the mechanisms behind it, as well as the impact of gut microbiota on intestinal homeostasis, are still sporadic. Functional gut microbiota analysis implies the metagenome studies annotating the relative genes' abundances within the microbial community, while transcriptomic, proteomic and metabolomic analysis enable the interpretation of their interaction with the host (10, 13).

These results provided the idea of using gut commensal bacteria as probiotics to restore a healthy homeostasis of the gastrointestinal tract in a natural way, and facilitated the development of Next-Generation Probiotics (14). The scientific community is increasingly becoming interested in studying and exploitation of Next-Generation Probiotics, especially those with health-promoting properties, whose ability to modulate the host's immune response was confirmed in scientific research. However, substantial work is needed to decipher the molecular mechanisms behind the role of gut microbiota in the amelioration of different diseases. For an in depth studying of these mechanisms,

the cultivation and characterization of predominantly anaerobic gut bacteria in still insufficiently known conditions is crucial and challenging. Hence, while probiotics have been considered safe for human consumption, a regulation on the safety status of Next-Generation Probiotics still does not exist. Due to the unregulated status of Next-Generation Probiotics, the use of postbiotics, referring to functional bioactive compounds, generated in a matrix during bacterial fermentation, which may be used to promote health (15), could be a more than welcome alternative. Some of the interesting candidates for Next-Generation Probiotics are *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, and *Bacteroides fragilis*.

Akkermansia muciniphila

By reviewing the articles dealing with the role of *A. muciniphila* as potential Next-Generation Probiotics, one can conclude this bacterium poses a conundrum, as lower abundances of *A. muciniphila* were correlated with different diseases, but some research has also correlated this bacterium with different pathological conditions. *A. muciniphila* was firstly isolated by Muriel Derrien from human feces as a strictly anaerobic Gram-negative bacterium, and the most abundantly present species in the human fecal microbiota (0.5-5% of total human fecal bacteria) (16). It was characterized as a bacterium using mucin as a carbon, nitrogen, and an energy source. *A. muciniphila*, as well as another species of this genus, *A. glycaniphila*, originating from the feces of python, are found to be the only representatives of *Verrucobacterium*. Karcher et al. found a large phylogenetic diversity of the *A. muciniphila* species in humans, grouping this bacterium into five distinct candidate subspecies (17). This study supported the idea that this species is human-specific, as it was isolated only from animals in captivity. Additionally, according to this study, different subspecies of *A. muciniphila* with functional differences (e.g., the presence of putative exo/lipopolysaccharide operon) could inhabit the human intestine, and this could be the source of somewhat non-uniform effects of this species in different host models. For now, there is little connection between strain-specific differences in genome and functional features.

A. muciniphila is related with obesity in 233,000 Google search results, pointing to the great interest in the role of this bacterium in the overweight state and conditions associated with it, such as diabetes (with 167,000 Google results) and other metabolic disorders. Obesity has been denoted as one of the biggest public-health issues in the 21st century (18). Besides being associated with various chronic diseases (e.g., diabetes type 2, osteoarthritis, tumors, cardiovascular and neuropsychiatric diseases), obesity represents a huge burden on the society, influencing the overall quality of life and often leading to the development of psychosocial disorders (19). In addition to lifestyle, gut microbes have been recognized as important in regulating host metabolism (20). Commonly, the Firmicutes/Bacteroides ratio positively correlates with an increase of body mass index (BMI) (21), but manipulation of specific bacteria abundance is viewed as very promising in preventing or treating obesity. Different studies investigated the potential of using live or pasteurized *A. muciniphila* in the treatment of obesity. By

supplementing C57BL/6 overweight mice fed by a high-fat diet (HFD) with pasteurized *A. muciniphila* (22), Yang et al. showed positive effects of this treatment on obesity parameters. These mice had a decreased caloric intake and consequently decreased the body weight gain. This treatment also improved glucose homeostasis and insulin sensitivity, reduced total fat and major adipose tissues weights, and led to the lowering of intestinal inflammation. Nevertheless, Everard et al. revealed that the effects could only be achieved by supplementation with live, but not with pasteurized bacteria (23). Plovier et al. (24) further challenged the effects achieved with live *A. muciniphila*, showing greater effects of pasteurized bacteria on the weight and fat mass gain in treated animals. This study showed a higher fecal caloric content in the experimental group fed with pasteurized *A. muciniphila*, implying that this treatment reduces caloric absorption. Finally, the treatment with Amuc_1100, an outer membrane protein of *A. muciniphila*, expressed in *E. coli*, resulted in lower fat mass and body weight. Ashrafian et al. also demonstrated that the same and even greater effects on body weight reduction could be achieved by supplementation of HFD-mice with extracellular vesicles produced by *A. muciniphila* in comparison to live bacteria (25). Interestingly, pasteurized *A. muciniphila* was approved by the European Food Safety Authority (EFSA) as a novel food, pursuant to the Regulation (EU) 2015/2283 (26). Plovier et al. further showed that the decrease in body mass in live bacteria treated mice was correlated with normolipemia and a reduction of insulin resistance, while the interaction of Amuc_1100 with Toll-like receptor 2 showed potential to improve gut barrier integrity (24). These effects implicated the potential role of *A. muciniphila* in Type 2 Diabetes (DT2), since impaired glucose tolerance, insulin resistance, obesity, abnormal lipid metabolism, and low-grade systemic inflammation are common manifestations of this disease (27). Indeed, a lot of literature data indicate a lower abundance of *A. muciniphila* in feces of DT2 patients (28–31). An improvement of the metabolism of glucose, lipids, and bile acid by *A. muciniphila* supplementation was proposed as the most important for preventive effects of this bacteria in DT2. The supplementation of mice with *A. muciniphila* increased glucagon-like peptide-1 (GLP-1) secretion and reduced the expression of glucose and fructose transporters in the gut, leading to a reduction in carbohydrate absorption, reduced adipose cell differentiation, and enhanced thermogenesis by upregulation of uncoupling protein 1 (Ucp1) (32), thus influencing body weight and composition (33). Different mechanisms of GLP-1 secretion control by *A. muciniphila* were proposed, such as stimulation by propionate production (34), and stimulation by the interaction of intercellular adhesion molecule 2 (ICAM-2) on immune cells and *A. muciniphila*-derived protein P9, leading to the activation of the phosphatidylinositol 3-kinase (PI3K)-Akt pathway implicated in glucose and lipid metabolism (35, 36). *A. muciniphila* degrades mucin and produces short-chain fatty acids (SCFA), polysaccharides, and indole derivatives, and all these compounds are shown to be involved in lipid metabolism. Lukovac et al. (37), showed that *A. muciniphila* is involved in the control of expression of fasting-induced adipose factor/angiopoietin-like protein (Fiaf/Angptl4), involved in the deposition of triglycerides in adipocytes (38). Furthermore, these authors published that *A. muciniphila* induces a

decrease of G protein-coupled receptor 43 (Gpr43), which activated with SCFAs regulates obesity and inflammatory diseases (39), and peroxisome proliferator-activated receptor gamma (Pparγ), involved in microbiota-induced expression of Fiaf (40). Additionally, *A. muciniphila* stimulated the expression of histone deacetylase 3 (HDAC3) and HDAC5 (41). HDACs have been shown to be correlated with a number of biological processes, such as the stimulation of interleukin-8 (IL-8) and inhibition of monocyte chemoattractant protein 1 (MCP-1) production by intestinal epithelial cells (IEC) (42), thus being important in regulating intestinal inflammation. The reduction of HDAC3 expression was described in tissues of patients suffering from inflammatory bowel disease (IBD) and this enzyme has been proposed to mediate commensal-microbiota interactions (43).

The involvement of *A. muciniphila* in colitis and IBD is controversial. Literature data revealed that supplementation with this species ameliorate IBD (reviewed in (44)). At other hand Ganesh et al., published that treatment with *A. muciniphila* aggravates IBD (45). As it was demonstrated for different microorganisms, several studies have assumed that various *A. muciniphila* strains exhibit strain-specific properties on the gut barrier integrity (46,47) implying the vital role of phenotypic studies in investigation of probiotic potential (45). Liu et al., showed the strain-specific properties of *A. muciniphila* on the regulation of gut epithelial barrier and revealed that this ability depends on the genes implicated in the cellular surface proteins synthesis (47). Strain ATCC BAA-835^T(=CIP 107961^T) has been used in most of the published articles.

Besides metabolic disease, *A. muciniphila* has become a hot topic in cancer research over the last few years (reaching 202,000 Google results). A low level of *A. muciniphila* was shown to be the main microbiota feature in mouse models of colorectal cancer (CRC) associated with colitis and in patients with CRC (48, 49). Furthermore, these studies demonstrated that the supplementation of mice CRC models or patients with Amuc_1100 or pasteurized *A. muciniphila* was able to delay the development of tumor, and that this effect correlates with the direction of the immune response towards the expansion of cytotoxic T lymphocytes in the colon and local lymph nodes, as well as with TLR-2 dependent differentiation of pro-inflammatory (M1) macrophages. Apart from immunomodulatory anti-tumor effects, *A. muciniphila* produces Amuc_1434, a Mucin2-degrading enzyme, highly expressed in mucinous CRC, and could protect p53, thus promoting apoptosis of cancer cells (50, 51). In addition to the effects of solo applied *A. muciniphila* in patients and in cancer mice models, a high number of studies pointed to the possible contribution of *A. muciniphila* application to the anti-cancer effects of different types of anti-cancer therapies, such as cisplatin (52, 53) and abiraterone acetate (54). The main focus on this species in cancer research was caused by results pointing to role in the success of anti-tumor therapy based on Immune Checkpoint Inhibitors (ICIs) that targets the PD-1/PD-L1 axis (55). In this research on patients with renal cell carcinoma (RCC), non–small cell lung cancer (NSCLC), and melanoma, Routy and colleagues found that antibiotics treatment correlated with shorter survival rate of NSCLC patients and even represented a good marker for PD-1 blockade resistance. Moreover,

they showed that *A. muciniphila* abundance in patients' feces at the moment of diagnosis was significantly related to promising clinical outcomes in NSCLC and RCC. In addition, the application of fecal microbiota transplantation (FMT) to avatar mice revealed that the resistance to PD-1 blockade depends on the microbiota, and could be overcome by orally applied *A. muciniphila*. This effect correlated with higher enrollment of CCR9+CXCR3+CD4+T lymphocytes into mouse tumor and was dependent on IL-12 production. Even though these results sound convincing, taking into consideration overall results dealing with the implication of the human gut microbiota in ICI response, no consensus on the exclusive role of *A. muciniphila* was observed, while other species like *Faecalibacterium prausnitzii* (56), *Bifidobacterium longum* (57), *Bacteroides caccae* (58) were shown to be important for ICI response. Furthermore, the investigation on the correlation of microbiota composition with the efficacy of dendritic cells-based anti-cancer vaccine *in vitro* pointed to a strong negative correlation of *A. muciniphila* abundance with the pro-inflammatory properties of differentiated dendritic cells and with their potential to induce differentiation of Th1 cells *in vitro*. Thus, the positive role of *A. muciniphila* should not be taken for granted and should be investigated in every type of tumor and in every type of anti-cancer therapy.

Although the exact role and the mechanism of action of *A. muciniphila* in the gut-brain axis has not been completely clarified, a vast amount of evidence points to its potential as a therapeutic target for brain function and disease. While only a few studies have evaluated the therapeutic effects of *A. muciniphila* applications in these disorders, Xu et al. gave a comprehensive review of the mechanisms of *A. muciniphila* in the gut-brain axis, including its protective effect on the intestinal epithelial barrier, immunomodulation and production of metabolites, like SCFAs, amino acids, and their derivatives (59). The results of Xu et al. suggested that *A. muciniphila* is involved in the production of SCFAs, primarily butyrate, considered to be histone deacetylases regulators (41), and recognized as molecules important for brain development and correlated with depression (60), schizophrenia (61), and Alzheimer's disease (62), pointing to the possible role of *A. muciniphila*-derived SCFAs in the microbiota-gut-brain axis. Dooling et al. showed that *A. muciniphila* reduced the gamma-glutamylation of amino acids and increased the ratio of hippocampal gamma-aminobutyric acid (GABA)/glutamate, preventing seizures (63). Oral supplementation of *A. muciniphila* in chronic restraint stress mice led to the restoration of corticosterone, dopamine, serotonin, and brain-derived neurotrophic factor levels, indicating its role in the regulation of hormones, neurotransmitters, and neurotrophic factors (64). Additionally, some evidence of the therapeutic potential of *A. muciniphila* in different neuropsychiatric disorders, such as Alzheimer's disease, multiple sclerosis, Parkinson's disease, and amyotrophic lateral sclerosis, was also reviewed (59).

Faecalibacterium prausnitzii

Faecalibacterium prausnitzii (*F. prausnitzii*) is a Gram-negative, non-sporeforming bacterium, belonging to the *Ruminococcaceae* family, phylum Firmicutes.

Based on a phylogenetic evaluation using 16S rRNA sequencing, the species *F. prausnitzii* can be divided into two phylogroups and three clusters (65). This obligatory anaerobic bacterium thrives in oxygen-free conditions. Despite being extremely oxygen sensitive, *F. prausnitzii* may tolerate the presence of oxygen if the media contain glutathione, cysteine, or flavins (66). It has been discovered that in healthy humans, *F. prausnitzii* represents more than 5% of fecal bacteria. Hence, it is thought to be one of the most abundant anaerobic bacteria in the human gut (67–69).

F. prausnitzii represents an important part of the gut microbiota with a huge impact on the host's health (70). Literature data showed that alterations in the abundance of *F. prausnitzii* are often correlated with dysbiosis, leading to various human disorders including ulcerative colitis, chronic idiopathic diarrhea, acute appendicitis, colorectal cancer, type 2 diabetes, obesity, atopic diseases, neuroendocrine tumors of the mid gut, liver transplantation, etc. (68, 71, 72). Its low incidence in many intestinal diseases, especially in IBD, Crohn's disease, coeliac disease, and irritable bowel syndrome (IBS), raises the possibility that it might serve as a marker for intestinal health (68). Additionally, the association between frailty in the elderly, which is linked to increased incidence of depressive disorder, and the diversity of the fecal microbiome was investigated (73).

Due to its impressive metabolic abilities, *F. prausnitzii* is found to be very important for the production of various metabolites with health benefits. Its capacity to metabolize a variety of carbohydrates, such as apple pectin, inulin, and various carbon sources from the host, is well documented (74–76). It generates SCFAs, particularly butyrate, during the fermentation process (77). Butyrate was shown to modulate immune responses and exert anti-inflammatory effects in the gut through its inhibition of NF-κB (78). Hence, it points to the immunomodulatory effects of *F. prausnitzii*, promoting anti-inflammatory responses and contributing to immune homeostasis in the gut.

According to the obtained scientific data, anti-inflammatory compounds originating from *F. prausnitzii* could be involved in protecting gut barrier integrity and reestablishing zona occludens 1 (ZO-1) expression under diabetic conditions, presumably via the tight junction pathway (79). Due to its ability to elicit high levels of IL-10 and low levels of IL-12 and IFN-γ production, an anti-inflammatory profile of *F. prausnitzii* was revealed (70). Furthermore, through excreted metabolites, *F. prausnitzii* is able to inhibit the activation of NF-κB and production of IL-8, factors involved in the inflammation process (80). In addition, the microbial anti-inflammatory molecule (MAM) originating from *F. prausnitzii* may inhibit the NF-κB pathway in IEC, preventing the occurrence of colitis in an animal model (81).

According to studies, *F. prausnitzii* can be engaged in cross feeding interactions with other gut microbiota members, e.g., *F. prausnitzii* is often balanced with the other main commensal bacterium *Bacteroides thetaiotaomicron,* since they are metabolically complementary in the sense that *B. thetaiotaomicron*-produced acetate is consumed by *F. prausnitzii, which in turn produces butyrate, allowing colonic epithelial homeostasis to be maintained by modifying goblet cells and mucin glycosylation (82).

Furthermore, *F. prausnitzii* has been linked to improved glucose metabolism, insulin sensitivity, and lipid metabolism. A negative correlation has been revealed between insulin resistance and *F. prausnitzii* (28). The obtained results revealed that *F. prausnitzii* may be an excellent candidate for a new treatment approach for type 2 diabetes, since it improves insulin resistance index (IR) and lipid metabolism, while lowering inflammation (83).

Additionally, research findings indicate that *F. prausnitzii* may be used as a psychobiotic due to its mental health benefit linked to the alleviation of anxiety and depression symptoms in rats (84). Based on the results of the microbiome analysis of the feces from patients with Alzheimer's disease and mild cognitive impairment (MCI), it can be concluded that *F. prausnitzii* has a positive correlation with cognitive scores in the MCI group in comparison to healthy subjects (85). Further experiments confirmed that two *F. prausnitzii* strains, live Fp360 and pasteurized Fp14, isolated from the healthy group, improved cognitive impairment in an Alzheimer's disease mouse model, indicating the possible application of *F. prausnitzii* in gut microbiome modulation in people suffering from Alzheimer's-type dementia.

A comprehensive study of *F. prausnitzii* and its metabolites in human feces may be useful in developing treatment options and personalized therapy. Available knowledge points to the potential significance of *F. prausnitzii* in maintaining gut function and host wellbeing. Although *F. prausnitzii* shows promise as a potential probiotic, more work is needed to completely reveal its mode of action, optimal dose, and safety issues. The challenges associated with its anaerobic nature and the need for further clinical studies limit its current availability as a stand-alone probiotic. Nonetheless, ongoing research in the field of gut microbiota and its potential therapeutic applications may shed more light on the benefits of *F. prausnitzii* as a candidate for Next-Generation Probiotics.

Bacteroides fragilis

Bacteroides fragilis (*B. fragilis*) is an obligate anaerobe usually found in the human gut, but it has also been detected in the upper respiratory and female genital tract. It is a Gram-negative bacteria, one of the most common members of the genus *Bacteroides* (Phylum Bacteroidetes, Class Bacteroidia, Order Bacteroidales, Family *Bacteroidaceae*) (86). The first discovery of *B. fragilis* was in infected patients, and it was thus isolated as a pathogen strain (87). *B. fragilis* was frequently found in individuals with a variety inflammatory disorders, such as: IBD, endocarditis, bacteremia, septicemia, as well as infections of the abdomen, skin, bone and joint, female reproductive tract, central nervous system and lower respiratory tract (88–94). Besides humans, this bacteria colonizes mucosal surfaces in the lower gastrointestinal tracts of various mammals, mainly infant sheep, beef, rabbits and pigs (95–98). *B. fragilis* is able to metabolize polysaccharides as sources of carbon and energy, and even though it is an obligate anaerobe, it could be tolerant of oxygen exposure (99). There have been reports stating that a number of variables, including physical health, drug use, lifestyle choices, but most importantly food, might influence the amount of *B. fragilis* in the gut. A diet

high in carbohydrates significantly affected the amount of this bacteria in several studies (100, 101). Other studies suggest that vitamin D has a positive correlation with the quantity of *B. fragilis* (102), while the intake of probiotic drinks containing *Lactobacillus casei* Shirota (103) or heat-killed *L. kunkeei* YB38 (104) reduces the amount of this bacteria in the gut.

Previous studies indicate that there are two kinds of *B. fragilis* strains: nontoxigenic *B. fragilis* (NTBF) that do not harbor or secrete *B. fragilis* toxin (BFT), and enterotoxigenic *B. fragilis* (ETBF) strains that have *bft* genes, coding *B. fragilis* toxin in their pathogenicity islands (BfPAI) (105, 106). NTBF strains are frequently thought of as advantageous commensal inhabitants that could compete with ETBF. By releasing specific favorable chemicals, one of which has been identified as polysaccharide A (PSA), these beneficial strains support intestinal health (107). PSA possesses zwitterionic properties (having both positive and negative charges on the sugar molecule), which is a rare feature among a few known bacterial polysaccharides (108, 109). Further experiments need to determine the mechanism of the delivery of PSA to host cells. One possible mechanism is secretion via outer membrane vesicles, which have components that form the outer membrane, such as phospholipids, proteins and polysaccharides (110).

It is interesting that a large portion of the genome of *B. fragilis* gives information for capsular polysaccharide synthesis, enabling this bacteria to produce at least eight distinct capsular polysaccharides (111). An essential basis for *B. fragilis* colonization and function in the human colon may be provided by the extensive and variable expression of surface polysaccharide combinations, since *B. fragilis* mutants lacking surface polysaccharide expression have problems colonizing the intestine. It is important to note that these mutants use an alternative way of restoring the expression of multiple capsular polysaccharides, leading to stable commensalism. (112). Besides, in their capsular surface *Bacteroides* incorporate polysaccharides and glycoproteins with L-fucose, which is a plentiful surface molecule of intestinal epithelial cells, leading to the coordinated expression of this surface molecule by the host and symbiont. Therefore, a *Bacteroides* mutant which cannot cover its surface with L-fucose is incapable of successfully colonizing the mammalian intestine in a competitive environment. (113).

Despite previous reports that *B. fragilis* was frequently detected in various diseases, it is reported as a commensal, with potential as a probiotic (114), due to its immunoregulatory and health-promoting effects. In experimental colitis-related mouse models, it can direct an anti-inflammatory response and provide protection (115, 116), showing similar effects in autoimmune encephalomyelitis (117), colorectal cancer (118), pulmonary inflammation (119) and asthma (120). Additionally, *B. fragilis* has been shown to have positive effects on graft-versus-host disease (GVHD), a serious complication following allogeneic hematopoietic cell transplantation (allo-HCT) and proinflammatory syndrome brought on by donor T cells. It was shown that the consumption of a *B. fragilis* isolate improved gut health by enhancing gut diversity and helpful commensal bacteria, while reducing proinflammatory bacteria. PSA-deficient *B. fragilis* failed to protect recipients from GVHD, even though the administration of live or

heat-killed *B. fragilis* significantly reduced GVHD. Additionally, the administration of *B. fragilis* increased intestinal tight junction integrity and SCFAs, particularly butyric acid and acetic acid, in gut tissues (121). Due to its immunomodulatory effects on the gut-brain axis, *B. fragilis* PSA could be used as promising agent for potential new treatments of multiple sclerosis (MS) (122). It appears that PSA is a potent modulator of neuroinflammation, since PSA given orally was able to protect against a lethal viral neuroinflammatory disease, like herpes simplex encephalitis (HSE). A possible mechanism could be that PSA binds and stimulates intestinal TLR2$^+$ plasmacytoid dendritic cells and B cells to secrete IL-10, followed by the induction of regulatory T cells producing IL-10 and IFN-γ, which together suppress pathogenic inflammatory monocytes and neutrophils to prevent encephalitis (123). It is interesting that changes in PSA expression in the gut lumen can cause an imbalance that may lead to peripheral systemic autoimmune disorders like human MS or EAE, and changing the gut microbiota's composition may enable to control the ratio of disease prevention to disease induction (124).

Taking into account their controversial status, like any other potential probiotic strains, different *B. fragilis* strains need to be evaluated systematically to determine whether they comply with the probiotic safety requirements. For example, *B. fragilis* strain ZY-312 was evaluated systematically, and it was shown that its metabolite profile closely resembles descriptions of *B. fragilis* in Bergey's manual and that it lacks BFT toxin, and both healthy and immune-deficient mice were used to demonstrate *in vivo* safety. This strain has 11 antibiotic resistance genes, but they are located on the chromosome, eliminating the risk of plasmid-mediated transfer of antibiotic resistance (125).

Finally, it is important to have in mind that research in this field is ongoing, and more studies are needed to fully understand the therapeutic potential and mechanisms of action of *B. fragilis* and its potential for Next-Generation Probiotics.

Conclusion

Although studies investigating the role of microbiota-gut-brain axis in neurodegenerative and psychiatric disorders have shown promise, their exact mechanisms of action are still not clear, and strong evidence-based treatments have not yet been developed. Hence, the isolation of novel gut microbiota members, their detailed safety and health-promoting characterization, as well as the identification of the metabolites produced by gut microbiota members involved in host-microbe interactions, are part of a fascinating journey leading to a full understanding of their role in health and diseases and shedding a new light on the field. Finally, the new knowledge acquired in this pioneering field will enable the development of novel Next-Generation Probiotics for prevention and treatment of various diseases, along with the development of effective diagnostic/prevention strategies based on detecting the presence/absence of key gut microbiota players/metabolites in the human gut microbiome before the symptoms of the disease occur.

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Probiotici sledeće generacije: crevne bakterije koje unapređuju zdravlje

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Kratak sadržaj

Poslednjih godina se veliki broj patoloških stanja i bolesti dovodi u vezu sa disbiozom crevne mikrobiote i promenama u njenom funkcionisanju. Razvoj savremenih metoda molekularne mikrobiologije, uključujući kulturomiku i integrativne pristupe kao što su sekvenciranje sledeće generacije, transkriptomska analiza dualne RNK sekvence i analiza metabolomike, omogućio je identifikaciju, kultivaciju i karakterizaciju novih anaerobnih, do sada nekultivisanih probiotika, nazvanih probiotici sledeće generacije. Pored toga, rezultati *in vitro* i *in vivo* studija proučavanja interakcija domaćina sa mikrobiotom pomogli su u rasvetljavanju mehanizama delovanja probiotika sledeće generacije. Na kraju, dobijeni podaci o probioticima sledeće generacije pomoći će da se prošire naučna saznanja o ovim bakterijama, kako u pogledu njihove bezbednosti, tako i u pogledu njihovog uticaja na zdravlje, otvarajući mogućnost za nove terapijske pristupe u prevenciji i terapiji metaboličkih bolesti, tumora, neurodegenerativnih i psihijatrijskih bolesti i drugih bolesti, u cilju ublažavanja simptoma bolesti i poboljšanja kvaliteta života pacijenata i njihovih porodica. Do sada najbolje opisani probiotici sledeće generacije su *Akkermansia muciniphila*, *Fecalibacterium prauznitzii* i *Bacteroides fragilis*.

Ključne reči: probiotici sledeće generacije, *Akkermansia muciniphila*, *Fecalibacterium prauznitzii*, *Bacteroides fragilis*